-> Histochemistry

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The Histochemical Demonstration of

CYTOCHROME OXIDASE

in Frozen Tissue Sections

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Not For Use In Diagnostic Procedures

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INTRODUCTION

There have been several methods published for the histochemical demonstration of Cytochrome Oxidase activity.[1-4] Burstone[2] has reviewed many of the procedures employed for detecting Cytochrome Oxidase activity in tissues. One of the methods[1] uses a combination of amines to obtain what has been termed the "most sensitive substrate." The procedure described in this bulletin is based on a technique described by Burstone.[1]

PRINCIPLE

A quinoline derivative, 8-amino-1,2,3,4-tetrahydroquinoline, is used as a substrate for tissue Cytochrome Oxidase. When acted upon by the enzyme, the quinoline derivative forms a blue dye through chelation with a metal. The reaction is augmented in the presence of added Cytochrome C.

REAGENTS

For Research Use Only
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A. p-AMINODIPHENYLAMINE, Stock No. PAD-15

Contains N-Phenyl-p-phenylene diamine (Variamine Blue RT Base), 15 mg capsule.

Store at room temperature.

CAUTION: Avoid contact with skin. Do not breathe dust.

B. 8-AMINO-1,2,3,4-TETRA-HYDROQUINOLINE, Stock No. 185-2

Store dark below 0°C.

C. TRIZMA® BUFFER, Stock No. 106-74

Contains Tris(hydroxymethyl)aminomethane, 0.2 mol/liter, pH 7.4 at 25°C, and chloroform added as preservative.

Store in the refrigerator at 0-5°C.

Suitable for use in the absence of visible microbial growth.

D. ACETATE BUFFER SOLUTION, Stock No. 105-52

Acetate, 0.2 M, pH 5.2 at 25°C, and chloroform added as preservative.

Store in the refrigerator at 0-5°C.

Suitable for use in the absence of visible microbial growth.

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E. COBALT(OUS) ACETATE, Stock No. 185-5

Contains $Co(CH_3CO_2)_2 \cdot 4\ H_2O$, preweighed 5-g packages.

Store at room temperature.

CAUTION: Avoid contact with skin. Do not breathe dust.

E'. COBALT-FORMALIN SOLUTION

Prepare the following mixture:

5 ml	Acetate Buffer, Stock No. 105-52
40 ml	Water
5 g	Cobalt(ous) Acetate, Stock No. 185-5
5 ml	Formalin (Reagent G)

Mix to dissolve.

Prepare fresh reagent weekly.

F. GLYCEROL-GELATIN, Stock No. GG-1

A Glycerol-Gelatin mixture containing 1% Phenol.

Store in the refrigerator at 0-5°C.

REQUIRED BUT NOT PROVIDED

G. FORMALIN, Reagent Grade, 40% Formaldehyde

H. ETHANOL, Reagent Grade

OPTIONAL

I. CYTOCHROME-C, TYPE III, Product No. C 2506

Cytochrome-C from horse heart.

Store dark and desiccated below 0°C.

SPECIMEN PREPARATION

Frozen sections 2-15 μ thick may be prepared from a variety of tissues and mounted on microscope slides using the Adamstone-Taylor cold knife technique.[3,4] Burstone[3] found that these mounted sections could be stored frozen for several days before use.

INSTRUMENT AND MATERIALS REQUIRED

Microscope, microscope slides or cover slips, staining dishes.

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PROCEDURE

1. Open a 50 mg ampule of 8-Amino-1,2,3,4-Tetrahydroquinoline, Stock No. 185-2, add 0.5 ml Ethanol (Reagent H) to dissolve contents and transfer solution to a 125 ml Erlenmeyer flask.

2. Add contents of one capsule p-Aminodiphenylamine, Stock No. PAD-15 and swirl to dissolve.

3. Add: 35 ml distilled water (solution becomes cloudy)
   15 ml Trizma® Buffer, Stock No. 106-74

   Shake well.

   Centrifuge and transfer supernatant fluid into a Coplin jar.

   NOTE: Filtration into a Coplin jar may be substituted for centrifugation.

4. Immerse mounted tissue sections in Coplin jar and incubate at room temperature for 15 minutes or longer.

5. Transfer mounted sections into another Coplin jar containing Cobalt-Formalin Solution (Reagent E').

   Allow tissue sections to undergo chelation-fixation for 1 hour or longer.

6. Wash slides in running tap water for 5-10 minutes.

7. Mount in Glycerol-Gelatin, Stock No. GG-1.

NOTE: For tissues with low activity, Burstone[1] suggests that the reaction mixture be augmented by the addition of 10-20 mg of Cytochrome-C after centrifugation or filtration (Step 3). Sigma Cytochrome-C, Type III, Product No. C 2506, may be useful for this purpose.

RESULTS

For the staining pattern observed with various tissues, it is suggested that the reader review the photographs in Burstone's[1] report.

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BIBLIOGRAPHY

1. Burstone MS: Modifications of histochemical techniques for the demonstration of cytochrome oxidase. J Histochem Cytochem 9:59, 1961
2. Burstone MS: Oxidases IN Enzyme Histochemistry, Academic Press, 1962, pp 429-448
3. Burstone MS: Histochemical demonstration of cytochrome oxidase with new amine reagents. J Histochem Cytochem 8:63, 1960
4. Burstone MS: Histochemical localization of oxidase activity in the mitochondria of the human heart. Nature 184:476, 1959

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TECHNICAL BULLETINS

Available without charge to qualified laboratories and schools

The following is a complete list of bulletins including those for in vitro diagnostic use, that are provided by SIGMA as of October, 1977.

ADENOSINE-5'-TRIPHOSPHATE (ATP) — Ultraviolet enzymatic procedure; for blood, at 340 nm. Employs phosphoglycerate kinase/glyceraldehyde phosphate dehydrogenase enzyme coupled system. ..... No. 366-UV

ALBUMIN — Colorimetric procedure; for serum, at 625-635 nm. Based on anion binding capacity of albumin for bromcresol green. ..... No. 630

ALCOHOL, ETHYL (ETHANOL) — Ultraviolet enzymatic procedure; for blood, serum or plasma, at 340 nm. ..... No. 331-UV

ALDOLASE — Colorimetric procedure; for serum, at 500-580 nm. Based on measurement of hydrazone derivatives of trioses formed from fructose-1,6-diphosphate. ..... No. 752

AMMONIA — Ultraviolet enzymatic procedure; for plasma, at 340 nm. Based on the conversion of α-ketoglutarate and ammonia to glutamate in the presence of glutamate dehydrogenase. ..... No. 170-UV

AMYLASE — Colorimetric procedure; for serum or urine. Based on visual observation of the starch-iodine color change. ..... No. 700

BILIRUBIN — Two colorimetric procedures; for serum or plasma, at 600 nm. Method A, for narrow-bandwidth spectrophotometers, is based on millimolar extinction coefficient of alkaline azobilirubin. Method B, for wide-bandwidth spectrophotometers, requires use of a bilirubin reference for calibration purposes. ..... No. 605

BIOLUMINESCENCE DEMONSTRATION PROCEDURE — Visual light is produced as a result of the reaction between luciferin, ATP and molecular oxygen, catalyzed by luciferase (uses desiccated firefly tails as source of luciferin and luciferase). For laboratory use only. ..... No. 2-FF

CALIBRATION PROCEDURE FOR WIDE BANDWIDTH SPECTROPHOTOMETERS — For reactions involving NADH and NADPH, at 340 nm. Describes a method for correcting wide bandwidth instrument readings for direct use in calculations. For laboratory use only. ..... No. 30-UV

CARBAMYL PHOSPHATE and/or ORNITHINE — Colorimetric procedure; at 490 nm. Measures citrulline formed by action of ornithine carbamyl transferase. For laboratory use only. ..... No. 22

CHLORIDE — Titrimetric procedure, Schales and Schales; for serum, plasma, urine or cerebrospinal fluid. ..... No. 830

CHOLINESTERASE — Colorimetric procedure; for serum or plasma, at 400-440 nm. Measures acetic acid liberated from acetylcholine. ..... No. 420

CREATININE — Colorimetric procedure; for serum, plasma or urine, at 490-510 nm. Modified Jaffe' reaction; avoids deproteinization. ..... No. 555

CREATINE PHOSPHOKINASE (CPK) — Colorimetric procedure; for serum, plasma or cerebrospinal fluid, at 620-700 nm. Measures inorganic phosphorus released from phosphocreatine. ..... No. 661

CREATINE PHOSPHOKINASE (CPK) — Colorimetric procedure; for serum or plasma, at 500-540 nm. Creatine released from phosphocreatine is reacted with α-naphthol and diacetyl to form a red complex. ..... No. 520

CREATINE PHOSPHOKINASE (CPK) — Ultraviolet kinetic procedure; for serum, plasma or cerebrospinal fluid, at 340 nm. Employs hexokinase/glucose-6-phosphate dehydrogenase coupled enzyme system. All reagents conveniently pre-standardized in a single vial. ..... No. 45-UV

CREATINE PHOSPHOKINASE (CPK) — Automated continuous flow colorimetric procedure; for serum, at 500-540 nm utilizing phosphocreatine and ADP. Measures released creatine with an orcinol-diacetyl reagent. ..... No. 911

CREATINE PHOSPHOKINASE (CPK) ISOENZYMES — Colorimetric procedure for detecting CPK isoenzymes, following electrophoresis on agarose gels. Based on reduction of tetranitroblue tetrazolium. ..... No. 715-EP

CREATINE PHOSPHOKINASE (CPK) ISOENZYMES — Fluorometric procedure for detecting CPK isoenzymes following electrophoresis on agarose gels. Based on the fluorescence of reduced NADP. ..... No. 745-EP

CYTOCHROME OXIDASE—Histochemical procedure; for frozen tissue sections. Based on the Burstone procedure. For laboratory use. ..... No. 185

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Price List No. 185

as of March 1978

Reagents for the Histochemical Demonstration of

CYTOCHROME OXIDASE

per Sigma Technical Bulletin No. 185

KIT

Kit No.	PRICE U.S. $	PRICE U.K. £	CONTENTS – STOCK NUMBERS Base PAD-15	Quinolin 185-2	Trizma Buffer 106-74	Acet. Buffer 105-52	Cobalt Acet. 185-5	Glycerol GG-1
185A	42.00	29.37	10 caps	10 x 50 mg	150 ml	50 ml	10 x 5 g	15 ml

NOTES: 1. Kit No. 185-A contains sufficient reagents for preparing ten separate Coplin jars of solution (10 x 50 ml).
2. Kit does not contain Formalin or Ethanol which are also needed. Please obtain locally.

INDIVIDUAL REAGENTS

Stock No.	Item	Quantity	PRICE U.S. $	PRICE U.K. £
PAD-15	p-AMINODIPHENYLAMINE (Base), 15 mg Capsule	10 caps	2.50	1.75
185-2	8-AMINO-1,2,3,4-TETRAHYDRO-QUINOLINE, Grade III	50 mg	3.00	2.10
106-74	TRIZMA BUFFER, 0.2 M, pH 7.4 at 25°C	150 ml	3.00	2.10
105-52	ACETATE BUFFER, 0.2 M, pH 5.2 at 25°C	50 ml	1.50	1.05
185-5	COBALT(OUS) ACETATE, 5 g preweighed package	10 x 5 g	4.00	2.80
185-100	100 g bulk package	100 g	5.00	3.50
GG-1	GLYCEROL-GELATIN, 15 ml plastic dropper bottle	15 ml	1.00	0.70

OPTIONAL REAGENT
(Not available in kit)

C 2506	CYTOCHROME C, TYPE III From Horse Heart	50 mg	6.20	4.34
		100 mg	9.25	6.47
		250 mg	21.00	14.69
		500 mg	38.50	26.93
		1 g	66.50	46.51

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2,3-DIPHOSPHOGLYCERIC ACID (2,3-DPG) — Ultraviolet enzymatic procedure; for erythrocytes, at 340 nm. Employs phosphoglycerate mutase/phosphoglycerate phosphokinase/glyceraldehyde phosphate dehydrogenase enzyme coupled system. ..... No. 35-UV

2,3-DIPHOSPHOGLYCERIC ACID (2,3-DPG) — Colorimetric procedure; for erythrocytes, at 620-700 nm. Measures phosphate released by action of phosphoglycerate mutase. ..... No. 665

ENDOTOXINS — An agglutination reaction for the detection of gram-negative bacterial endotoxins. For laboratory use only. ..... No. 210

FETAL HEMOGLOBIN — Histochemical acid elution procedure; for blood smears. ..... No. 285

FIBRIN DEGRADATION PRODUCTS (FDP) — A visual semi-quantitative estimation; in serum, by the staphylococcal cell suspension agglutination technique. ..... No. 850

FORMIMINO-L-GLUTAMIC ACID (FIGLU) — Ultraviolet enzymatic procedure; for urine, at approximately 365 nm. ..... No. 365-UV

GALACTOSE-1-PHOSPHATE URIDYL TRANSFERASE — Ultraviolet procedure; for erythrocytes, at 340 nm. Measures rate of consumption of uridine diphosphoglucose (UDPG). ..... No. 600-UV

GALACTOSE-1-PHOSPHATE URIDYL TRANSFERASE DEFICIENCY — Fluorescence screening procedure; for erthrocytes. Rate of NADPH formation is noted by visual observation of increasing fluorescence under long-wave ultraviolet (black) light. ..... No. 195

GLOBULIN, TOTAL — Colorimetric procedure; for serum, at 550-570 nm. Based on the modification of Hopkins-Cole reaction for tryptophan. ..... No. 560

GLUCOSE — Colorimetric enzymatic procedure; for serum or plasma, at 520 nm. Based on the use of hexokinase and glucose-6-phosphate dehydrogenase enzymes, coupled with the reduction of iodonitrotetrazolium to produce a colored INT formazan. ..... No. 115

GLUCOSE — Colorimetric procedure; for blood, plasma or serum, at 425-475 nm. Employs glucose oxidase and peroxidase with o-dianisidine as chromogen. ..... No. 510

GLUCOSE — Colorimetric procedure; for blood, plasma or serum, at 620-650 nm. Based on o-toluidine color reaction. ..... No. 635

GLUCOSE — Ultraviolet enzymatic procedure; for serum, plasma, cerebrospinal fluid and urine, at 340 nm. Employs hexokinase/glucose-6-phosphate dehydrogenase coupled enzyme system. All reagents conveniently pre-standardized in a single vial. ..... No. 15-UV

GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G-6-PD) — Semiquantitative colorimetric procedure; for erythrocytes. Based on visual observation of dichlorophenol indophenol dye decolorization. ..... No. 400

GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G-6-PD) DEFICIENCY — Fluorescence screening procedure; for erythrocytes. Rate of NADPH formation is noted by visual observation of increase in fluorescence under long wave ultraviolet light. ..... No. 202

β-GLUCURONIDASE — Colorimetric procedure; for serum or urine, at 550 nm. Based on measurement of phenolphthalein liberated from phenolphthalein mono-β-glucuronic acid. ..... No. 325

γ-GLUTAMYL TRANSPEPTIDASE (GGTP) — Colorimetric procedure; for serum or plasma, at 545 nm. Based on measurement of p-nitroanaline liberated from γ-glutamyl-p-nitroanilide. ..... No. 545

γ-GLUTAMYL TRANSPEPTIDASE (GGTP) — Kinetic procedure; for serum or plasma, at 405 nm. Based on rate of formation of p-nitroaniline from γ-glutamyl-p-nitroanilide. ..... No. 415

GLUTATHIONE REDUCTASE DEFICIENCY — Fluorescence screening procedure; for erythrocytes. Rate of NADPH consumption is noted by visual observation of loss in fluorescence under long wave ultraviolet light. All reagents conveniently pre-standardized in a single vial. ..... No. 190

"GOOD" BUFFERS — A series of amine buffers with a pH range of 5.5-11.0. For laboratory use only. ..... No. 107

HEMOGLOBIN — Colorimetric procedure; for whole blood, at 540 nm. Employs modified Drabkin's solution and a stable, freeze dried methemoglobin standard. ..... No. 525

HEPARIN — A quantitative clotting procedure; for plasma. Based on heparin's effect on activated Factor X (Xa) inhibitors. ..... No. 870

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α-HYDROXYBUTYRATE DEHYDROGENASE (α-HBD) — Ultraviolet kinetic procedure; for serum, at 340 nm. ..... No. 20-UV

α-HYDROXYBUTYRATE DEHYDROGENASE (α-HBD) — Colorimetric procedure; for serum, at 400-550 nm. Based on measurement of hydrazone derivative of residual substrate, α-ketobutyrate. ..... No. 495

INORGANIC PHOSPHORUS — Colorimetric procedure; for serum or urine, at 620-700 nm. Based on the Fiske and SubbaRow procedure. ..... No. 670

IRON AND TOTAL IRON-BINDING CAPACITY — Colorimetric procedure; for serum, at 560 nm. Based on the reaction of ferrous ions with ferrozine to produce a magenta colored complex. ..... No. 565

ISOCITRATE DEHYDROGENASE (ICD) — Colorimetric procedure; for serum, at 380-400 nm. Based on measurement of hydrazone derivative of α-ketoglutarate formed from isocitrate. ..... No. 176

ISOCITRATE DEHYDROGENASE (ICD) — Ultraviolet kinetic procedure; for serum, at 340 nm. ..... No. 153-UV

17-KETOSTEROIDS (17-KS) — Colorimetric procedure; for urine, at 520-550 nm. Based on the Zimmermann reaction. ..... No. 17

17-KETOSTEROIDS (17-KS) — Colorimetric procedure; for urine, at 480, 520 and 560 nm. Based on a simplified, rapid extraction and improved Zimmermann reaction. ..... No. 270

L(+)LACTIC ACID — Ultraviolet enzymatic procedure; for blood, at 340 nm. ..... No. 826-UV

LACTATE DEHYDROGENASE (LDH) — Colorimetric procedure; for serum, urine or cerebrospinal fluid, at 400-550 nm. Based on the Berger-Broida method. ..... No. 500

LACTATE DEHYDROGENASE (LDH) — Ultraviolet kinetic procedure; for serum, at 340 nm. Based on method of Wacker et al. ..... No. 225-UV

LACTATE DEHYDROGENASE (LDH) — Ultraviolet kinetic procedure; for serum, at 340 nm. Based on the Wroblewski-LaDue method. ..... No. 340-UV

LACTATE DEHYDROGENASE (LDH) ISOENZYMES — Colorimetric procedure following electrophoresis on cellulose acetate or agarose gels. Based on the reduction of tetranitroblue tetrazolium. ..... No. 705-EP

LEUCINE AMINOPEPTIDASE (LAP) — Colorimetric procedure; for serum and urine, at 540-620 nm. Modification of the Goldbarg and Rutenburg procedure. ..... No. 251

LIPASE — Titrimetric procedure; for serum (Sigma-Tietz method). Based on titration of liberated fatty acids from a standardized olive oil substrate. ..... No. 800

MALATE DEHYDROGENASE (MDH) — Ultraviolet kinetic procedure; for serum, at 340 nm. Based on the Siegel-Bing method. ..... No. 340-UV

NBT BLOOD TEST — Histochemical procedure; for blood smears. Based on reduction of NBT to highly colored formazan deposits by leukocytes in the presence of bacterial infection. ..... No. 840

5'-NUCLEOTIDASE — Colorimetric procedure; for serum, at 660 nm. Based on the Dixon and Purdom method. ..... No. 675

ORNITHINE and/or CARBAMYL PHOSPHATE — Colorimetric procedure; at 490 nm. Measures citrulline formed by the action of ornithine carbamyl transferase. For laboratory use only. ..... No. 22

ORNITHINE CARBAMYL TRANSFERASE (OCT) — Colorimetric procedure; for serum, at 400-420 nm. Nesslerization of ammonia formed from decomposition of carbamyl arsenate. ..... No. 108

PHENYLALANINE — Fluorometric procedure; for serum, at 365 nm activation, 450-535 nm emission. Based on the McCaman and Robins method. ..... No. 60-F

PHENYLALANINE — A microbiologic screening procedure for early detection of phenylketonuria based on the Guthrie inhibition assay. ..... No. 160

PHOSPHATASE — Colorimetric procedure; for serum, at 400-420 nm, for alkaline, acid and prostatic acid phosphatase. Also urinary alkaline phosphatase. Based on the Bessey-Lowry-Brock procedure. ..... No. 104

PHOSPHATASE, ACID — Histochemical procedure; for blood or bone marrow smears. For laboratory use only. ..... No. 385

PHOSPHATASE, ALKALINE — Histochemical procedure; for blood or bone marrow smears. ..... No. 85

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PHOSPHATASE, ALKALINE — Kinetic colorimetric procedure; for serum or plasma, at 410 nm. Requires a narrow-bandwidth spectrophotometer. Based on measurement of liberated p-nitrophenol. ..... No. 244

PHOSPHATASE ISOENZYMES, ALKALINE — Colorimetric procedure for detection of alkaline phosphatase isoenzymes following electrophoresis on agarose gels. Based on the formation of an indigo dye. ..... No. 710-EP

PHOSPHATASE, MILK — Colorimetric procedure; for milk, at 400-420 nm. For laboratory use only. ..... No. 104M

PHOSPHOHEXOSE ISOMERASE — Colorimetric procedure; for serum, at 470-510 nm. Based on the Bodansky method. ..... No. 650

PHOSPHOHEXOSE ISOMERASE — Ultraviolet kinetic procedure; for serum, at 340 nm. ..... No. 355-UV

PROTEIN, TOTAL — Colorimetric procedure; for serum, at 540-545 nm. Based on the Biuret reaction. ..... No. 540

PYRUVATE KINASE DEFICIENCY — Fluorescence screening procedure; for erythrocytes. Rate of NADH consumption is noted by loss in fluorescence under long-wave ultraviolet light. All reagents conveniently pre-standardized in a single vial. ..... No. 205

PYRUVIC ACID — Ultraviolet enzymatic procedure; for blood, at 340 nm. ..... No. 726-UV

SORBITOL DEHYDROGENASE (SDH) — Ultraviolet kinetic procedure; for serum, at 340 nm. β-D-Fructose used as substrate. ..... No. 50-UV

TRANSAMINASE (GOT and GPT) — Colorimetric procedure; for serum, plasma or cerebrospinal fluid, at 490-520 nm. Based on the Sigma-Frankel method. ..... No. 505

TRANSAMINASE (GOT and GPT) — Ultraviolet kinetic procedure; for serum, plasma or cerebrospinal fluid, at 340 nm. Based on the Karmen et al. method. All reagents conveniently pre-standardized in a single vial. ..... No. 55-UV

TRANSAMINASE (GOT and GPT) — Ultraviolet kinetic procedure; for serum, plasma or cerebrospinal fluid, at 340 nm. Based on the Karmen et al. method. A two-reagent system for the LKB Reaction Rate Analyzer or similar automated equipment. ..... No. 155-UV

TRIGLYCERIDES — Colorimetric procedure; for serum or plasma, at 405-415 nm. Based on the Kessler-Lederer and Fletcher methods. ..... No. 405

TRIGLYCERIDES — Ultraviolet semi-enzymatic procedure; for serum or plasma, at 340 nm. Based on assay of glycerol released from triglycerides after saponification. ..... No. 320-UV

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TYROSINE — Fluorometric procedure; for serum, at 460 nm excitation and 570 nm emission. Based on the reaction of tyrosine with 1-nitroso-2-naphthol. ..... No. 70-F

UREA NITROGEN — Colorimetric procedure; for serum, plasma or urine, at 515-540 nm. Based on the urea-diacetyl monoxime reaction. ..... No. 535

UREA NITROGEN — Colorimetric procedure; for serum, plasma or urine, at 500-650 nm. Ammonia formed by action of urease is determined by Berthelot reaction. ..... No. 640

UREA NITROGEN — Ultraviolet enzymatic procedure; for serum, plasma or urine, at 340 nm. Employs urease/glutamate dehydrogenase coupled enzyme system. All reagents conveniently pre-standardized in a single vial. ..... No. 65-UV

URIC ACID — Colorimetric procedure; for urine, at 650-750 nm. Combines the accuracy of the enzyme uricase with the simplicity of a colorimetric method. ..... No. 680

URIC ACID — Ultraviolet procedure; for serum, or urine, at 292 nm. Based on specific reaction of uric acid with uricase. ..... No. 292-UV

VANILMANDELIC ACID (VMA) — Colorimetric procedure; for urine, at 470-510 nm. Based on method of Sunderman et al. ..... No. 480

VANILMANDELIC ACID (VMA) — Ultraviolet procedure; for urine, at 360 nm. Simplification of VMA Procedure No. 480 if instrument is available with 360 nm reading capability. ..... No. 481-UV

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